



WHAT IS CLAIMED IS:

A fusion protein, comprising a nucleotide binding domain operatively linked to a ligand binding domain derived from an intracellular receptor, wherein:

5

the nucleotide binding domain is a polydactyl zinc-finger peptide or modular portion thereof that specifically interacts with a contiguous nucleotide sequence of at least about 3 nucleotides; and

the fusion protein is a ligand activated transcriptional regulator.

10

- The fusion protein of claim 1, further comprising an 2. operatively linked transcription regulating domain.
- The fusion protein of claim 1, wherein the intracellular receptor is a nuclear hormone receptor.
- The fusion protein of claim 3, wherein the ligand binding domain derived from a nuclear hormone receptor has been modified to change its ligand selectivity compared to the native hormone receptor.
- The fusion protein of claim 4, wherein the modified ligand-5. binding domain is not substantially activated by endogenous ligands.
- The fusion protein of claim 1, wherein zinc-finger peptide 6. binds to a sequence of nucleotides of the formula (GNN), where G is quanidine, N is any nucleotide and n is an integer from 1 to 6.
 - 7. The fusion protein of claim 6, wherein n is 3 to 6.

wal The fusion protein of claim 1, wherein the zinc-finger peptide is comprised of modular units from a C2H2 zinc-finger peptide or a variant thereof that specifically interacts with a sequence of nucleotides and targets the fusion protein to a exogenous or endogenous gene that comprises the sequence of nucleotides.

- 9. The fusion protein of claim 1, wherein the zinc finger peptide is comprised of at least one zinc finger or a variant thereof that specifically binds to a targeted nucleic acid molecule.
- The fusion protein of claim 9, that comprises at least three 10. zinc fingers or variants thereof.

25

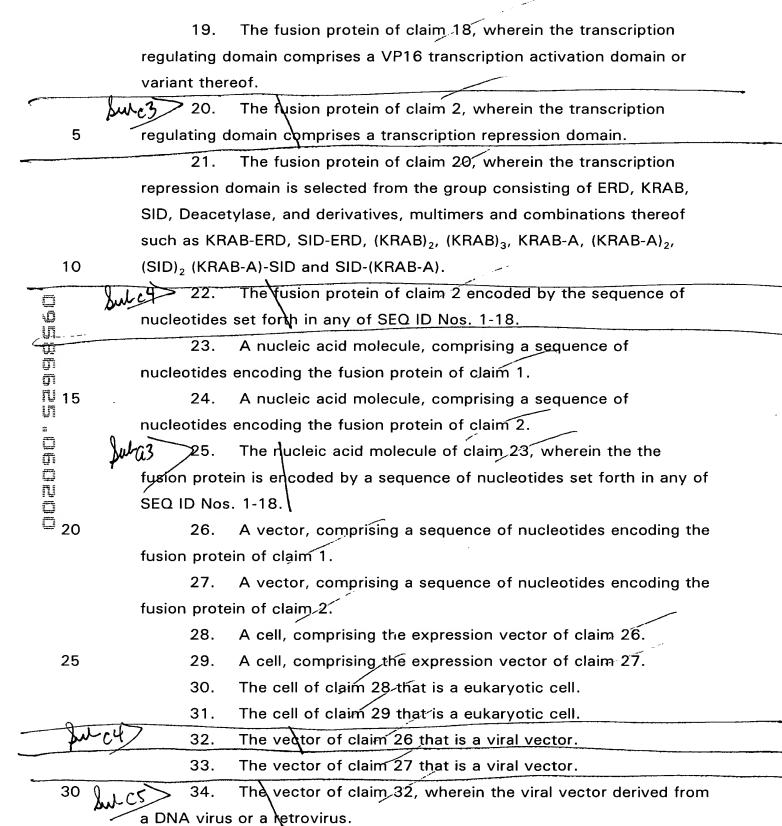
20

10





- 11. The fusion protein of claim 1, wherein the intracellular receptor is a nuclear hormone receptor selected from the group consisting of estrogen receptors, progesterone receptors, glucocorticoid-α receptors, glucocorticoid-β receptors, mineralocorticoid receptors, androgen receptors, thyroid hormone receptors, retinoic acid receptors, retinoid X receptors, Vitamin D receptors, COUP-TF receptors, ecdysone receptors, Nurr-I receptors and orphan receptors.
- 12. The fusion protein of claim 1, wherein the intracellular receptor is a steroid receptor.
- 13. The fusion protein of claim 4, wherein the hormone receptor is a progesterone receptor variant or an estrogen receptor variant, wherein a receptor variant comprises a ligand binding domain that has selectivity and sensitivity for endogenous and exogenous ligands that differ from its native ligands.
- 14. The fusion protein of claim 2, wherein the transcription regulating domain comprises a transcription activation domain.
- 15. The fusion protein of claim 2, wherein the transcription regulating domain comprises a transcription activation domain selected from the group consisting of VP16, VP64, TA2, STAT-6, p65 and derivatives, multimers and combinations thereof that have transcription activation activity.
- 16. The fusion protein of claim 14, wherein the transcription regulating domain comprises a nuclear hormone receptor transcription activation domain or variant thereof that has transcription activation activity.
- 17. The fusion protein of claim 14, wherein the transcription regulating domain comprises a steroid hormone receptor transcription activation domain or variant thereof.
- 18. The fusion protein of claim 14, wherein the transcription regulating domain comprises a viral transcription activation domain or variant thereof that has transcription activation activity



The vector of claim 33 that is a viral vector. 36.

The vector of claim 36, wherein the viral vector derived from a DNA virus or a retrovirus.

The vector of claim 37 that is selected from the group 38. consisting of an adenoviral vector, and adeno-associated viral vector, a herpes virus vector, a vaccinia virus vector and a lentiviral vector.

DJBBBBB BBB

5

A combination, comprising: 39.

a fusion protein of claim 1 or a nucleic acid molecule comprising a sequence of nucleotides that encodes the fusion protein; and

a regulatable expression cassette that comprises at least one response element recognized by the nucleic acid binding domain of the fusion protein.

40. The combination of claim 39, wherein the cassette comprises a gene that encodes a therapeutic product.

41. The combination of claim 39 that comprises a single composition that contains the fusion protein or nucleic acid molecule that encodes the fusion protein, and the regulatable expression cassette in a pharmaceutically acceptable excipient.

The combination of claim 39, wherein the fusion protein or 42. nucleic acid molecule comprising a sequence of nucleotides that encodes the fusion protein, and the regulatable expression cassette are in separate compositions.

Jul c7 43. A composition for regulating gene expression comprising: an effective amount of the fusion protein of claim 1 or a nucleic acid molecule comprising a sequence of nucleotides that encodes the fusion protein; and

a pharmaceutically acceptable excipient.

25

20

15

Ħ



The composition of claim of claim 43 that is formulated for single dosage administration.

Sucs 5

- A composition for regulating gene expression comprising: 45. an effective amount of the fusion protein of claim-2; and a pharmaceutically acceptable excipient.
- The combination of claim 39, wherein the regulatable expression cassette comprises 3 to 6 response elements.
- A method for regulating gene expression in a cell, comprising:

introducing into a cell a fusion protein of claim 1 or a nucleic acid molecule that comprises a sequence of nucleotides that encodes the fusion protein; and

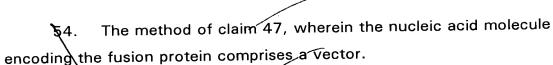
contacting the cell with a ligand that interacts with the binding domain in the fusion protein, whereby the fusion protein interacts with a target nucleic acid molecule to activate or repress transcription of a gene encoded by the fusion protein.

- The method of claim 47, wherein the ligand binding domain 48. is modified whereby it interacts with a non-natural ligand.
- The method of claim 47, wherein the target nucleic acid 49. molecule is endogenous to the cell.
- The method of claim 47, wherein the target nucleic acid 50. molecule is introduced to the call as part of an expression cassette.
- The method of claim 47, wherein the expression cassette and fusion protein or nucleic acid encoding the fusion protein are introduced at the same time.
- The method of claim 47, wherein the expression cassette 52. and fusion protein or nucleic acid encoding the fusion protein are introduced sequentially.
- The method of claim 47, wherein ligand is delivered to the 53. cell after the fusion protein or nucleic acid molecule encoding the fusion protein is introduced into the cell.

20

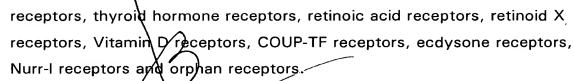
25

5



- 55. \setminus The method of claim 54, wherein the vector is a viral vector.
- 56. The method of claim 47, wherein the cell is in a mammal.
- 57. The method of claim 50, wherein the expression cassette is contained in a vector.
 - 58. The method of claim 57, wherein the vector is a viral vector.
 - 59. The method of claim 50, wherein the cell is in a mammal.
- 60. The method of claim 47, wherein the ligand binding domain derived from a nuclear hormone receptor has been modified to change its ligand selectivity compared to the native hormone receptor.
- 61. The method of claim 60, wherein the modified ligandbinding domain is not substantially activated by endogenous ligands.
- 62. The method of claim 47, wherein zinc-finger peptide binds to a sequence of nucleotides of the formula (GNN)_n, where G is guanidine, N is any nucleotide and n is an integer from 1 to 6.
 - 63. The method of claim 62, wherein n is 3 to 6.
- 64. The method of claim 47, wherein the zinc-finger peptide is comprised of modular units from a C2H2 zinc-finger peptide or a variant thereof that specifically interacts with a sequence of nucleotides and targets the fusion protein to a exogenous or endogenous gene that comprises the sequence of nucleotides.
 - 65. The method of claim 47, wherein the zinc finger peptide is comprised of at least one zinc finger or a variant thereof that specifically binds to a targeted nucleic acid molecule.
 - 66. The method of claim 65, that comprises at least three zinc fingers or variants thereof.
 - 67. The method of claim 47, wherein the intracellular receptor is a nuclear hormone receptor selected from the group consisting of estrogen receptors, progesterone receptors, glucocorticoid-α receptors, glucocorticoid-β receptors, mineralocorticoid receptors, androgen

20



- 68. The method of claim 47, wherein the intracellular receptor is a steroid receptor.
- 69. The fusion protein of claim 1, wherein the polydactyl zincfinger pentide or modular portion thereof specifically interacts with a contiguous nucleotide sequence of at least about 3 nucleotides to about 18 nucleotides.
- 70. A non-viral delivery system, comprising the fusion protein of claim 1 or a nucleic acid molecule encoding the fusion protein.
- 71. The non-viral delivery system of claim 70, further comprising a nucleic acid molecule that comprises an expression cassette containing a sequence of nucleotides with which the nucleic acid binding domain of the fusion protein interacts.
- 72. The non-viral delivery system of claim 70, wherein the non-viral delivery system is selected from the group consisting of DNA-ligand complexes, adenovirus-ligand-DNA complexes, direct injection of DNA, CaPO₄ precipitation, gene gun techniques, electroporation, liposomes and lipofection.
- V3. The fusion protein of claim 9, wherein the zinc finger peptide comprised of at least one zinc finger or a variant thereof specifically binds to a targeted nucleic acid molecule with a dissociation constant of less than about 1.0 nanomolar.

